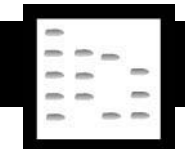
CHROMATOGRAPHY: LIQUID



Column Technology

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Introduction

Today, the main columns for high performance liquid chromatography (HPLC) are of tubular design, normally manufactured from high quality stainless steel and packed with spherical ceramic particles. Termination of the columns is via a metal frit held in place by an end fitting that can accept a 1/16 in (15.9 mm) male fitting to connect the column to the HPLC instrument.

Figure 1 shows a typical conventional HPLC column. This is now a mature design that has evolved over a period of 20 years. In that time, many variations have evolved, such as cartridge columns, removable end fitting columns, radial compression columns and nonmetal columns. Today, the most common analytical column has an inner diameter (i.d.) of 4.6 mm with an outer diameter of 1/4 in (63.5 mm). However, in recent years there has been a movement to smaller i.d. columns such as 3, 2, 1 and 0.5 mm. There has also been a development of packed capillary columns having i.d. of 0.32, 0.25 and 0.1 mm. Even newer columns with i.d. 0.05 mm have been developed for capillary electrochromatography (CEC). Columns are defined in **Table 1** according to their internal diameters.

The most common length of an HPLC column is 25 cm but analytical columns are available in lengths down to 3 cm and guard columns are commonly 1 cm long.

The material packed into columns is mainly silica based, spherical in shape with a particle size of 5 μm. Material of diameter 10 μm has now virtually disappeared from analytical columns and 3 μm material, although introduced in the mid-1980s, is still not widely used. Newer, smaller-diameter particles of 2 and 1 μm are also available but at the time of writing occupy a niche market only.

Figure 1 Typical HPLC column.

The Conventional Column

Conventional analytical columns are 4.6 or 4 mm i.d., 1/4 in (63.5 mm) o.d. They are manufactured from high quality stainless steel such as 316 to withstand the high pressures that are used in packing (up to 10 000 psi; 69 000 kPa) and in operating the columns (around 2500 psi; 17 000 kPa). Manufacturers take particular care with the inner bores of these tubes; some use tubing 'as-drawn', while others use electropolished tubing. There have been reports of chemical modification with silanes to the inner surface.

The column packing material is held in the column with an end frit, which in turn is held in place by the column end fitting. The frit is normally made from a sintered metal, the porosity being determined by the size of the packing material used. For 5 μm materials, a 2 μm porosity frit is normally used, but for 3 μm and smaller, a 0.5 μm frit is common. The most common type of end fittings are those manufactured by Swagelok, Parker and Valco. Typical end fittings are shown in **Figure 2**.

Frits are held on the column by compression fittings; although it is possible to remove the end fitting to repair or top up the packed material (or to clean or repair a damaged frit), it is not generally recommended as it is not usually possible to regain good performance. Inserting and removing these classical columns from an HPLC instrument cannot be done without tools. In an attempt to make this into a simple 'no tools operation', and to reduce the amount of hardware used in the tube section of the column, the cartridge column was developed. The first successful commercial cartridge column was designed by Brownlee Labs and is today still known as the Brownlee system.

In this system, the column design is very simple in that the tubular column has the packing material held in place by frits that are pushed into the end of the tube. This packed tube is held in the cartridge column

Table 1 Definitions of columns

Column type	*Internal diameter (mm)*
Analytical	20–3
Microbore	2–0.5
Capillary	< 0.5

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Figure 2 Typical end fittings.

held by hand-tight end nuts that are part of and free to rotate with the cartridge holder.

Since the Brownlee system was introduced, there have been many copies on this basic design and also developments into the finger-tight removable end fitting column (**Figure 3**). These columns, as shown in **Figure 4**, again have the frit pushed into the tube or held in place by a frit cap. This frit cap also contains a seal that allows the end fitting to be screwed down onto the frit. This design has many advantages. It allows easy removal of the frit to replace it or top up the column packing material, but more importantly,

Figure 3 Finger-tight end fitting column showing frit cap.

Figure 4 Removable end fitting column.

it allows columns to be coupled together with very low dead volumes.

Apart from the cartridge and removable end fitting columns, a different design approach was taken by Waters with their radial pack systems. As mentioned previously, high pressures are used to pack particles into column to obtain a well-packed bed. In packing columns, voids in the packing bed are produced if insufficient pressures are used; these voids are commonly along the axial direction. The idea of the radial packed column is to pack the material into a soft-walled tube. This tube is held in a device that can apply radial pressure, and so reduce the radial voids. Some examples are shown in **Figure 5**. This radial compression system has not been applied down to small i.d. tubes, but has been developed for semipreparative systems. In preparative systems, direct axial compression on to the top of the packing is also used.

Columns down to 3 mm i.d. can be used with what is commonly classed as conventional HPLC equipment, with detector flow cells in the order of 8 μL.

Figure 5 (See Colour Plate 21) Radial compression column cartridge.

Table 2 Volumetric flow rates required to maintain a linear flow velocity of 1.5 mm s^{-1} in columns of different diameters

Column diameter (mm)	*Flow rate (mL min^{-1})*	*Solvent consumption (mL h^{-1})*
1.0	0.04	2.4
2.0	0.18	10.8
3.0	0.39	23.4
4.0	0.7	42.0
4.6	**0.93**	**55.8**
5.0	1.1	66.0
8.0	2.81	168.6

Reducing the column diameter below 3 mm requires detectors with smaller volumes to reduce detector band broadening. Pumps that can produce lower flow rates are also required. **Table 2** gives the volumetric flow rates required to maintain a linear flow velocity of 1.5 mm s^{-1}.

Although microbore columns became commercially available in the mid-1980s, they have not displaced 4.6 mm i.d. columns. Many comparison reviews were written in the 1980s of 1 mm versus 4.6 mm i.d. columns. In general the conclusion was reached that solvent saving and packing material costs did not justify the capital expenditure in new equipment and that mass sensitivity enhancements were difficult to achieve. Since then much has improved, both in the instruments and especially in the column design, particularly in the end fittings used on < 3 mm i.d. columns.

From 1990 onward, further research was conducted and there was widespread discussion of the advantages of microbore columns, including increased resolution, decreased solvent consumption, lower heat capacity, increased mass sensitivity and, most importantly, the easier coupling of these columns to secondary systems and mass spectrometers. Columns with i.d. down to 1 mm are conventionally still manufactured from stainless steel, but as the i.d. is reduced so can the wall thickness be reduced. This allows the use of other materials, as the resulting pressure forces in the small i.d. tubes is also reduced. For 2 and 1 mm columns, glass-lined tubing has been used. This is in an attempt to give a very smooth inner surface, which in turn helps column packing and hence efficiency. Columns are now available manufactured from PEEK (poly(ether ether ketone), an ICI polymer). Columns of this material are produced in diameters down to 0.5 mm.

A conventional PEEK column is shown in **Figure 6**, and a 0.5 mm flexible PEEK column is shown in **Figure 7**. These columns have no wetted metal components and, since the 0.5 mm columns are flexible,

Figure 6 PEEK column.

for the first time in HPLC it has become possible to consider a different design of instrument, in that there is no longer any need to have a fixed distance between the injector and detector. Another feature of these columns is that they can be cut to any length. If the

Figure 7 PEEK-o-bore column.

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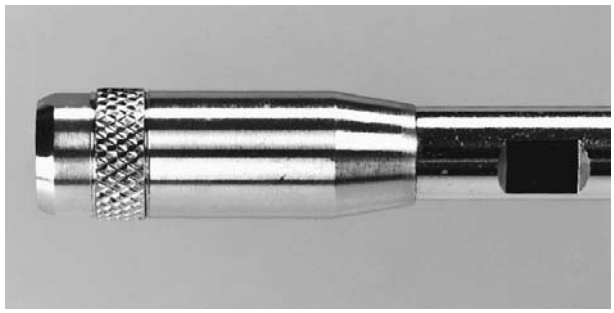

Figure 8 Integral guard column.

inlet blocks or becomes contaminated, then this part of the column can be cut off.

Capillary columns of less than 0.5 mm i.d. are almost exclusively manufactured from fused silica, in some cases with a metal outer case onto which the end fittings can be connected.

Guard Columns

As the name suggests, guard columns are used to protect the main analytical column. They are normally 1 cm in length, of the same diameter as the main column, and made of the same material. They are not extensively used in all application areas, possibly because they can reduce the total column efficiency and are not easy to install or replace. This is set to change with the development of removable end fitting columns. New designs, as shown in **Figure 8**, now allow easy connection and replacement of guard systems. However, manufacturers still only offer generic packings of either normal phase or reversed phase for these columns. **Figure 9** shows a guard column cartridge system that can be connected to any form of column.

Packing Materials

Porous silica is the most widely used adsorbent in HPLC, although extensive work has been conducted with alumina, zirconia and polymer supports. The majority of these supports are spherical and, for analytical applications with small molecules, have general physical parameters of pore size 10 nm, pore volume 0.5 mL g^{-1} and surface area 250 m^2 g^{-1}. The most common particle size used in columns is 5 μm. Care must be taken when using the particle size given by a manufacturer. What is meant by 5 μm particles, is actually a 5 μm particle size distribution. Depending upon the type and mode of measuring instrument used by the manufacturer, there will be a particle size distribution based on number, area or volume.

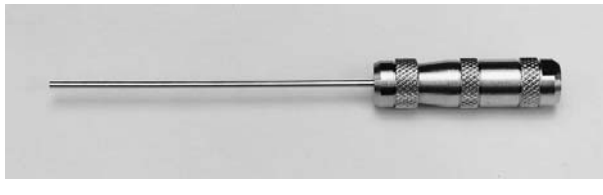

Figure 9 Stand-alone guard column.

In the number distribution the percentage of the particles in each size range is determined by dividing the number in each range by the total number of particles. In the area distribution, the percentage of the surface area in each size range is determined by dividing the surface area calculated for each size range by the total surface area of all the particles. This has the effect of emphasizing the larger particles more than the number distribution. For example, one particle of 10 μm diameter has the same surface area as 100 particles of 1 μm diameter. The volume distribution is calculated from the percentage of the volume of each particle size divided by the total volume of all the sizes. The volume distribution emphasizes the large particles even more than the area distribution, e.g. a 10 μm diameter particle has the same volume as 1000 particles of 1 μm. Typical distributions (for a 5 μm Spherisorb) are given in **Figure 10**, showing the emphasis on the larger particle sizes in the distributions for area and volume.

Silica particles used for packing are commonly manufactured from a sol–gel type process, starting from a cation-based sol. This produces gels that are high in their cationic species, for example Spherisorb has a sodium content of 1500 ppm. Newer manufacturing methods introduced in recent years, use organic tetraethyl orthosilicate (TEOS) condensation systems that can produce exceptionally pure silicas.

In the sol–gel process, the size of the particles can be controlled by the viscosity of the mixture, the emulsification rate and the amount and type of surfactant used to stabilize the emulsion. However, none of the processes can produce monodispersed particles – they produce a particle size distribution and the required particle size is then obtained from this by air classification or elutriation.

The tighter the particle size distribution, the more uniform a column packing can be made. However, with very tight particle size distributions it becomes more difficult to pack the columns. Van Deemter curves demonstrating the effect of particle size on the column efficiency are shown in **Figure 11**.

The surface area, pore size and pore volume of packing material are determined either by nitrogen BET or mercury porosimetry. As with particle size distributions, care must be used in the interpretation of these data because different models are used by

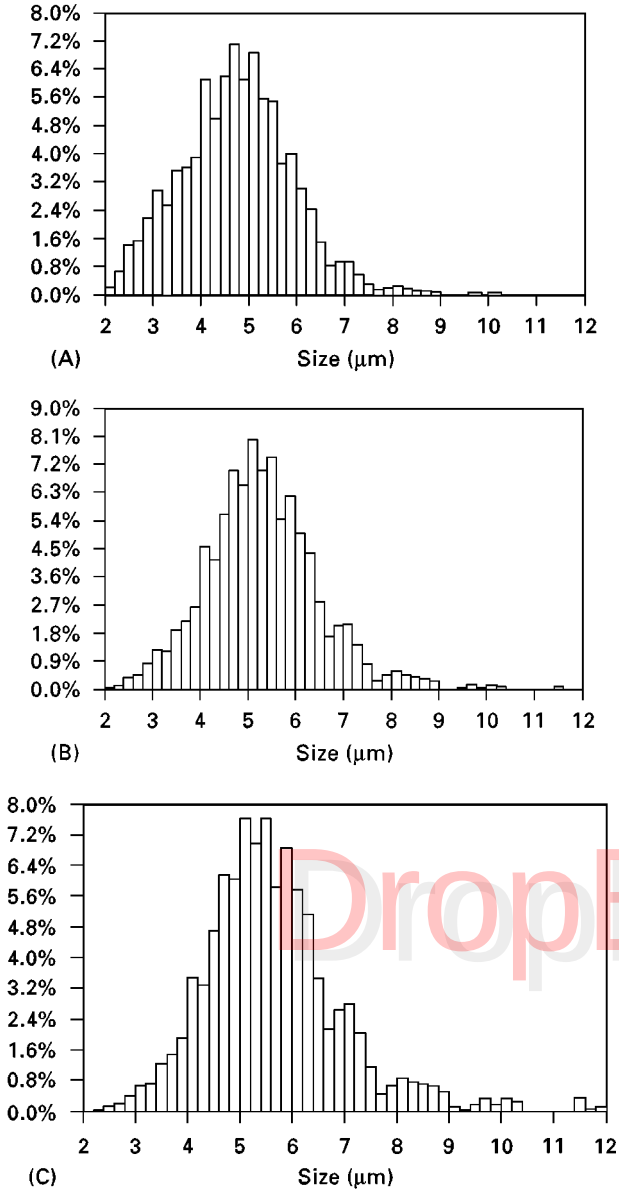

Figure 10 Particle size distributions for a 5 μm Spherisorb. (A) Number; (B) area; (C) volume.

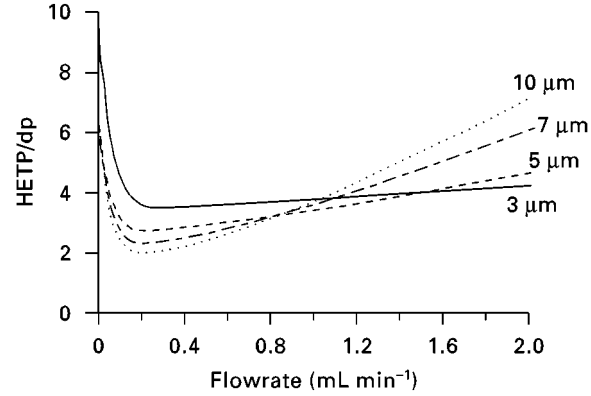

Figure 11 Van Deemter curves.

different manufacturers, which can impose different interpretations on the type and shape of pores. In HPLC, an important factor is the absence of micropores and mesopores. Micropores are defined as having a diameter of less than 1 nm, while mesopores are less than 5 nm in diameter. If these pores are included in the surface area calculations, then very large surface area values are obtained. However, most analyte molecules cannot penetrate into micropores and even if they could possibly penetrate into the mesopores, diffusion is very restricted by steric hindrance, which in turn affects mass transfer and hence efficiency.

Silica is a preferred adsorbent in HPLC as the surface characteristics can be modified by chemical reaction to change the hydrophilic silica surface into a hydrophobic surface suitable for reversed-phase chromatography, or to place an ionic exchange material on the surface for ion exchange chromatography.

A very broad range of functionality can be attached to the silica surface, through reaction with organosilanes, the most popular being C_{18} or octadecylsilane. The linkage onto the silica is of the type Si–O–Si–R, where R is the functional group. Manufacturers use a variety of silanes, ranging from monochloro to trichlorosilanes to mono and tri methoxy and ethoxy silanes. Attachment of the monofunctional silane onto the surface of the silica leads to a monomeric phase, while the addition of small amounts of water into the bonding process can lead to polymeric phases.

Manufacturers normally give bonding figures as % carbon loadings. This is a very misleading figure as the percentage of carbon loading alone is not a relevant parameter because of differences in the surface area of the original silica, which result in different surface densities of the bonded alkyl groups. **Table 3**

Table 3 Column packing parameters

Description	*Column volume (mL)*	*Si density (g mL $^{-1}$)*	*Mass of packing (g)*	*Carbon load (%)*	*Amount of carbon in the column (g)*
Nova-Pak C_{18}	1.8	0.91	1.64	7	0.12
μBondapak C_{18}	1.8	0.46	0.82	10	0.08

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Table 4 Some commercially available bonded phases

Phase name	*Usage*	*Comments*
C_1	Purification	
C_4	Protein separations	
C_6	Protein separations	
C_8	General purpose	Almost 80% of all applications have been developed with C_8 and C_{18} phases
C_{18} ODS	General purpose	Almost 80% of all applications have been developed with C_8 and C_{18} phases
Phenyl	Separation of complex mixtures	Induced dipole interactions with polar analytes
Cyano CN	Broad spectrum of mixtures with different polarities	Can be used in both normal and reversed phase
Amino NH_2	Sugar analysis and for aromatic compounds	Weak anion exchanger
Diol	Complex mixtures	Slightly polar adsorbent for normal phase
Ion exchange	Ionic species	
Chiral phases	Enantiomer separations	Four major types available: donor, polymer, cavity and exchange

ODS, octadecylsilica.

outlines this problem, showing the amount of carbon on a material against its percentage carbon loading.

Table 4 provides a broad summary of the bonded phases that are commercially available. It must be stressed that this is in no way an extensive list. There are many specialized phase–column combinations supplied by many manufacturers. In particular, these speciality phases tend to be in the chiral separation areas.

The term end-capping was introduced in the late 1970s to indicate that a secondary treatment had been applied to a bonded phase. This was an attempt to cover all available silanols. The most common end-capping agent is trimethylchlorosilane; this molecule is relatively small and so can penetrate into the pore system of the adsorbent. Generally end-capping leads to an improvement in the chromatography of basic compounds. However, even after the best end-capping, it is estimated that about 50% of all silanols are still unbonded.

A term that has recently been introduced into column packings is 'base-deactivated' reversed phases. This is a particularly unfortunate term since these packings are not deactivated with bases but have surface treatments or treatments of the silica that make them particularly useful for separating basic compounds. Amines, for example, tend to tail on conventional reversed-phase column packings because of the residual acidic silanols. These base-deactivated packings are normally manufactured from the new pure silicas and have a more uniform or reduced level of silanols.

A major problem with silica is the small pH range over which the adsorbent can be used. Above pH 8.5 the siloxane bridge is broken, leading to breakdown through solvation of the particle. Below pH 2.5, the bonded phase is hydrolysed from the surface of the silica. In an attempt to extend the pH range of silica, attempts have been made to manufacture pH-stable bondings. These have normally been based on coating the silica with a polymer, such as polybutadiene or a grafted polysiloxane.

Other ceramics have been reported as packing materials. The most common are alumina, zirconia and titania. Although it is possible to attach hydrophobic groups onto all of these materials, with alumina the silane is very easily removed from the surface. To obtain a hydrophobic character for alumina, polybutadiene or grafted polysiloxane polymers are used. These types of bondings have been divided into two groups. The first comprises those polymers with siloxane bonds such as polymethyloctylsiloxane, polymethyloctadecylsiloxane and silica monomers, while the second is represented by purely organic monomers and oligomers such as polybutadiene.

Another support that has been developed and can be used under very aggressive conditions is porous graphitic carbon.

Polymer-based adsorbents are also used as a support material in HPLC columns. They are manufactured from synthetic cross-linked organic polymers. Their main application area is in size-exclusion chromatography and ion exchange chromatography. In normal-phase and reversed-phase HPLC they still have very few applications. A problem is that even though the new polymer packings are highly cross-linked and stable, they still carry the history of their precursors and they can swell with some solvents.

Conclusions

Much has changed over the last few years in both the manufacture of support materials and the design of columns. Manufacturers of supports now make more reproducible materials and very pure forms of support materials, especially in silica, where metal

impurity levels are now less than 5 ppm (the first silicas used had typical impurity levels of 1000–2000 ppm). In the silica support materials the latest developments have been towards base-deactivated materials and have tended to focus on the new very pure silicas. Particle size distributions have also become tighter around given means, leading to more stable and reproducible columns.

In column design, the move has been to cartridge type systems having smaller tube i.d. with smaller particles and shorter lengths.

The focus on development is now moving to micro systems. Future developments are likely to be systems with smaller and smaller column i.d., and even complete columns on microchips.

See Colour Plates 21, 22.

See also: **II/Chromatography: Liquid:** Mechanisms: Reversed Phases. **III/Porous Graphitic Carbon: Liquid Chromatography. Porous Polymers: Liquid Chromatography**.

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DropBooks

Countercurrent Liquid Chromatography

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Introduction

Countercurrent chromatography (CCC) belongs to the family of liquid partition chromatography but with one distinct feature: the system totally eliminates the use of a solid support. Unlike liquid chromatography (LC), CCC utilizes two immiscible solvent phases. The partition process takes place in an open column where one phase (the mobile phase) continuously passes through the other (stationary phase), which is permanently retained in the column. To retain the stationary phase within the column, the system uses effective combinations of the column configuration and a force field (gravitational or centrifugal). Hence CCC instruments display a variety of forms that are quite different from those used in LC.

Because no solid support is used, CCC can eliminate all the complications arising from the use of a solid support such as adsorptive sample loss and denaturation, tailing of solute peaks and contamination.

The Two Basic CCC Systems

All existing CCC systems have been developed from two basic forms, the hydrostatic equilibrium system and the hydrodynamic equilibrium system (**Figure 1**).

The basic hydrostatic system (left) uses a stationary coiled tube. The coil is first filled with the stationary phase (either the lighter or the heavier phase of an equilibrated two-phase solvent system) and the mobile phase is introduced from one end of the coil. Owing to the action of gravity, the mobile phase percolates through the segment of the stationary phase in one side of the coil. This process continues until the mobile phase reaches the other end of the coil. Thereafter the mobile phase only displaces the same phase leaving the stationary phase in the coil. Consequently, solutes introduced at the inlet of the coil are partitioned between the two phases in each helical turn and separated according to their partition coefficients.

The basic hydrodynamic system (right) uses a similar arrangement except that the coil is rotated around its own axis. This simple motion produces a profound effect on the hydrodynamic process in the coil by generating an Archimedean screw force. All